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A genetic locus regulates the expression of tissue-specific mRNAs from multiple transcription units

(endogenous retroviral gene/GIX phenotype/gp70/RNA blot hybridization/transcriptional control)

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ABSTRACT **129 GIX$^-$ mice, unlike animals of the congeneic partner strain GIX$^+$, do not express significant amounts of the retroviral antigens gp70 and p30. Evidence is presented indicating that the GIX phenotype is specified by a distinct regulatory gene acting on multiple transcription units to control the levels of accumulation of specific mRNA species. The steady-state levels of retroviral-homologous mRNA from the tissues of GIX$^+$ and GIX$^-$ mice were examined by blot hybridization using as probes DNA fragments from cloned murine leukemia viruses. RNA potentially encoding viral antigens was reduced or absent in GIX$^-$ mice, even though no differences in integrated viral genomes were detected between these congeneic strains by DNA blotting. Tissue-specific patterns of accumulation of these RNA species were detected in brain, epididymis, liver, spleen, and thymus, and several distinct RNA species were found to be coordinately regulated with the GIX phenotype. Measurements of RNA synthesis suggest a major role for transcriptional control in the regulation of some retroviral messages.**

Genomes of laboratory mice contain multiple copies of DNA sequences homologous with murine leukemia virus (MLV). The expression of these viral genes is subject to undefined host controls, resulting in strain-specific patterns of viral production (1). Unlike many strains, the 129 mouse does not produce infectious virions from endogenous viral information (2). Only the major envelope glycoprotein (gp70) and the major core protein (p30) of MLV have been detected in 129 tissues (3). The first demonstration of a retroviral gene product in these mice was the detection of GIX (4), which was found to be a type-specific antigenic determinant of gp70 (5–7). Classically, the GIX antigenic determinant is considered characteristic of the thymic stage of T-cell differentiation in many murine strains (4). Other gp70 proteins have been demonstrated in a variety of murine tissues of other differentiation pathways, including spleen, liver, epididymis, serum, and bone marrow (3, 8–11), and in a number of nonviral tumors (refs. 12 and 13; unpublished results). The GIX determinant is inherited as a mendelian trait (4, 14), a property that allowed the derivation of a congeneic strain that lacks this thymic determinant (15).

The GIX serological determinant is also present on some viruses and has recently been shown to correlate with the absence of a complex carbohydrate chain near the carboxyl terminus of gp70 (16) that is the result of a single nucleotide difference between the genomes of GIX$^+$ and GIX$^-$ virus (17). However, several features suggest that, in this nonviral case, the difference between GIX$^+$ and GIX$^-$ 129 congeneic mice is not the result of differential glycosylation. This GIX$^-$ phenotype has been shown to result from the absence of gp70 molecules (7, 8, 18) rather than the obscuring of an antigenic determinant by glycosylation. Another gp70 determinant (GERLD), which may represent a distinct gp70 molecule, is also lost in the 129 GIX$^-$ mouse (19). Also, a corresponding decrease (8) or absence (3) of p30 accompanies the GIX$^-$ phenotype in 129 mice, suggesting that the expression of an entire retroviral genome is being affected.

The loss of the expressed retroviral genome of 129 mice (i.e., the structural genes for gp70 and p30), as has been suggested (3, 18), would also result in a GIX$^-$ phenotype. However, several observations suggest instead that a *trans*-acting regulatory gene that promotes gp70 expression in 129 GIX$^+$ has been lost or inactivated in the congeneic 129 GIX$^-$ strain. First, genetic analysis has identified two unlinked genes (*Gv-1* and *Gv-2*), one dominant and one codominant, that are required for GIX expression, only one of which (*Gv-1*) is absent from GIX$^-$ mice, the strains being congeneic at *Gv-2* and at all other loci tested (14). Second, the gp70 molecules found at different tissue sites of 129 GIX$^+$ mice but absent or reduced in 129 GIX$^-$ mice are serologically distinct (9) and have distinct tryptic peptide maps (20), suggesting that multiple structural genes are being affected. Third, although 129 GIX$^-$ mice are often described as completely lacking gp70, low but detectable quantities of this antigen have been reported (8). The analysis of this genetic regulation has been limited to such serological and biochemical studies of the protein antigens. In this report, we describe nucleic acid hybridization analyses that support the conclusion that 129 GIX$^+$ and GIX$^-$ mice differ in the expression of the same complement of retroviral genes. The GIX phenotype correlates with the differential accumulation of specific mRNA molecules homologous with retroviral DNA, implicating a regulatory locus that coordinately controls the expression of a number of distinct retroviral transcripts.

MATERIALS AND METHODS

Mouse Strains and Cell Lines. 129 GIX$^+$ and 129 GIX$^-$ congeneic mouse stocks were obtained from the Congenic Mouse Production Facility of Memorial Sloan–Kettering Cancer Center through the generosity of E. A. Boyce and maintained at the Scripps Clinic and Research Foundation vivarium. A lymphoblastoid cell line chronically infected with a murine leukemia virus (Scripps Clinic and Research Foundation 60A) was maintained as a suspension culture in RPMI 1640 medium/10% fetal bovine serum.

Isolation of Chromosomal DNA and Restriction Analysis. Liver, thymus, and sperm were obtained from 4- to 6-wk-old 129 GIX$^+$ and 129 GIX$^-$ male mice. DNA was extracted from thymus and liver nuclei and from sperm after treatment with

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Abbreviations: MLV, murine leukemia virus; MoLV, Moloney leukemia virus; kb, kilobase(s).

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proteinase K/NaDodSO$_4$. DNA was analyzed by the method of Southern (21) and hybridized with ^{32}P-labeled nick-translated probes (22) at a specific activity of 0.8–2 × 10^9 cpm/μg.

RNA Isolation and Blot Hybridization. Mouse tissues were obtained from 4- to 8-wk-old males, frozen immediately in liquid nitrogen, and stored at −70°C until use. Frozen tissues were pulverized by pounding in a stainless steel press at −70°C, blended with proteinase K (250 μg/ml) and 1% NaDodSO$_4$ in isotonic buffer, extracted with phenol/chloroform, and precipitated with ethanol. RNA was extracted from tissue culture cells after lysis in 0.25% Nonidet P-40 in isotonic buffer (23). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (24), fractionated on denaturing formaldehyde agarose gels (25), and transferred to nitrocellulose (26) as described (27).

Recombinant DNA Clones. A recombinant DNA clone of Moloney leukemia virus (MoLV) in the plasmid pBR322 (pMLV-1) was obtained from I. Verma (28). Subgenomic fragments and subclones were prepared by using standard procedures utilizing a restriction map deduced from the complete nucleotide sequence (29) and are illustrated in Fig. 1. Clone pAKV$^{gag\text{-}pol}$ of the *gag-pol* region of AKV-1 was kindly provided by D. Lowy. Probes for constitutively synthesized mRNA transcripts (pCHO B and pCHO C) were obtained through the generosity of M. Harpold (30). The recombinant plasmid DNA was purified essentially as described by Clewell and Helinski (31). All procedures for growth of bacteria and handling of recombinant DNA were in accordance with the National Institutes of Health guidelines for recombinant DNA research.

***In Vitro* Transcription in Isolated Nuclei and RNA Hybridization.** Nuclei were isolated and incubated with [α-^{32}P]UTP as described (32–34). In some experiments, α-amanatin was included during UTP incorporation at 1 μg/ml. Labeled RNA was incubated with single-stranded recombinant plasmid DNA immobilized on nitrocellulose filters at 67°C for 48 hr in 0.3 M NaCl/20 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid/10 mM EDTA/0.2% NaDodSO$_4$, pH 7.4.

RESULTS

129 GIX$^-$ Mice Are Congeneic with GIX$^+$ for Endogenous Retroviral Genomes. Although no infectious retroviral particles have been isolated from mouse strain 129, viral-cDNA is detectable in 129 cellular DNA (1, 35, 36). To determine whether the difference in the presence of viral proteins detected between strain 129 and its congeneic 129 GIX$^-$ partner strain represented a change in viral structural genes, the sequences complementary to viral *env* in the chromosomal DNA of these strains were examined by DNA blot hybridization (21). Retroviral sequences were detected in the germ-line and the somatic-cell DNA of both 129 GIX$^+$ and the congeneic GIX$^-$ mice. These sequences are present in high molecular weight DNA (Fig. 2*A*) and therefore do not reflect the presence of unintegrated viral cDNA. Furthermore, no differences were detected in the number or location of viral sequences between the germline and somatic DNA (Fig. 2*B*). This result indicates that no infection of the somatic tissue with exogenous viruses or rearrangements of endogenous sequences has taken place.

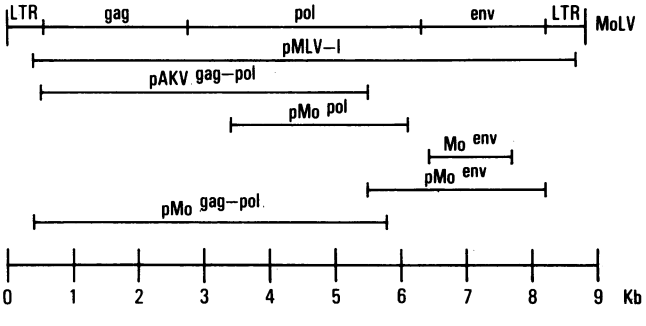

FIG. 1. Linear map of MLV recombinant DNA probes. pMLV-1 (28) is presented corresponding to the gene order of viral genomic RNA. pMo^{pol}, pMo^{env}, pMo$^{gag\text{-}pol}$, and Mo^{env} were derived from pMLV-1 following digestion with *Kpn* I, *Pvu* II, *Xba* I, and *Hpa* I, respectively. pAKV$^{gag\text{-}pol}$, a subclone from AKR ecotropic virus, was kindly provided by D. Lowy. LTR, long terminal repeat.

A minimal estimate of the number of separately integrated retroviruses can be derived from an *Eco*RI digestion pattern because cleavage at unique sites in the flanking DNA generates a distinct DNA fragment for each integrated MLV (37, 38). However, a comparison of 129 GIX$^+$ and GIX$^-$ DNA homologous with MoLV *env* (Fig. 2*B*) reveals that the lack of viral antigens in GIX$^-$ is not reflected in a corresponding difference in the number of viral structural genes. All *Eco*RI-generated viral DNA fragments of antigen-expressing 129 GIX$^+$ mice are also present in the DNA of the nonexpressing 129 GIX$^-$ mice. A similar analysis of *gag-pol* homologous sequences (not shown) detected no differences between GIX$^+$ and GIX$^-$ mice.

mRNA Levels Reflect the GIX Phenotype. The expression of endogenous retroviral genes was examined in the epididymis of GIX$^+$ and GIX$^-$ 129 mice because the male genital tract is the major site of accumulation of gp70 (10). To measure steady-state levels of mRNA potentially coding for gp70, total poly(A)$^+$ RNA was fractionated by molecular weight on a denaturing agarose gel and transferred to nitrocellulose. Hybridization with MoLV DNA (Fig. 3*A*) showed that the reduced expression of the GIX antigen in 129 GIX$^-$ epididymis correlates with a reduced accumulation of retroviral RNA. Whereas the 8.0-, 7.2-, and 3.5-kilobase (kb) RNA species present in a viral infected cell are also present in the normal 129 GIX$^+$ epididymis, a greatly reduced amount of these molecules is observed in the 129 GIX$^-$ epididymis.

Hybridization of the GIX$^+$ and GIX$^-$ epididymal RNA preparations with the gp70-coding region of MoLV revealed that the RNA species potentially encoding the gp70 protein is reduced in GIX$^-$ mice (Fig. 3*B*). During a normal viral infection, a 21S subgenomic RNA is translated into the envelope polyprotein precursor (40). This molecule probably corresponds to the 3.5-

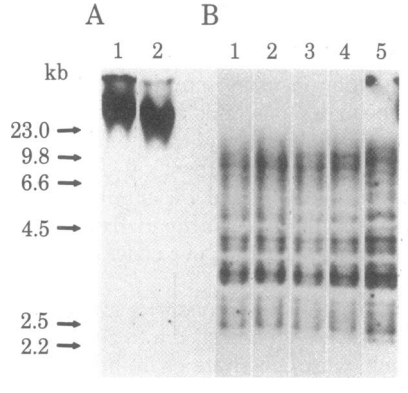

FIG. 2. Endogenous MoLV *env* homologous sequences in strain 129 mice. High molecular weight DNAs from liver and thymus of GIX$^+$ and GIX$^-$ 129 mice and from sperm of GIX$^-$ mice were separated on a 0.6% agarose gel untreated (*A*) and after digestion with *Eco*RI (*B*). After transfer to nitrocellulose, the DNA was annealed to nick-translated Mo^{env} DNA fragment at 67°C in 0.75 M NaCl and the blot was washed at 50°C with 30 mM NaCl and exposed to Kodak XAR-5 film with an intensifying screen. The mobility of *Hin*dIII restriction fragments of λ DNA is indicated. (*A*) Lanes: 1, GIX$^+$ liver; 2, GIX$^-$ thymus. (*B*) Lanes: 1, GIX$^+$ liver; 2, GIX$^+$ thymus; 3, GIX$^-$ liver; 4, GIX$^-$ thymus; 5, GIX$^-$ sperm.

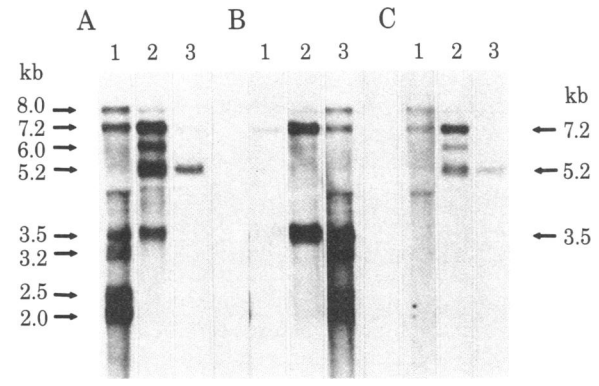

FIG. 3. Hybridization of polyadenylylated RNA from SCRF 60A cells and 129 GIX$^+$ and 129 GIX$^-$ epididymis with retrovirus probes. Poly(A)$^+$RNA (5 μg) was electrophoresed in triplicate lanes of a 1% agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized with the nick-translated recombinant DNA probes (2–4 $\times$ 10^8 cpm/μg) pMLV-1 (*A*), Mo^{env} (*B*), and pAKV$^{gag\text{-}pol}$ (*C*). Sizes of individual bands were estimated from the mobility of fragments of known size generated by restriction endonuclease digestion of pMLV-1. Preliminary data (not shown) indicate that the 2.5- and 2.0-kb RNAs in 60A cells contain gp70-coding information and the U3 polyadenylylation site but lack the intervening p15E coding information normally present in the 21S transcript just upstream of U3. Thus, these molecules may arise from a specific splice that removes p15E sequences and may encode a secreted form of gp70 since p15E normally functions as the membrane anchor for gp70 in infected cells and viruses (39). (*A* and *C*) Lanes: 1, 60A; 2, GIX$^+$; 3, GIX$^-$. (*B*) Lanes: 1, GIX$^-$; 2, GIX$^+$; 3, 60A.

kb band from 60A cells and epididymis, which contains Mo^{env}-homologous sequences (Fig. 3*B*), and sediments in a sucrose gradient at approximately 22 S (data not shown). In GIX$^-$ RNA, the 3.5-kb species is virtually undetectable with a probe to the entire MoLV but can be visualized in low amounts with a probe specific for the *env* gene (compare Fig. 3 *A* and *B*). The low but detectable amount of *env*-related RNA in GIX$^-$ epididymis supports the conclusion that the GIX$^-$ phenotype is not a result of the absence of the structural gene for gp70 but is some alternative genetic event that affects the cellular accumulation of viral RNA.

To further characterize the retroviral RNA species regulated by *Gv-1*, epididymal RNA from 129 GIX$^+$ mice was hybridized with probes specific for the *pol* region of MoLV (not shown) and the *gag-pol* coding region of cloned AKV (Fig. 3*C*). The 8.0-kb and 7.2-kb bands, which are present in reduced amounts in the GIX$^-$ epididymis, have sequences homologous to all the subregions represented in the viral probes and probably correspond to full-length viral transcripts. The 3.5-kb band, which is also reduced in GIX$^-$, is homologous to only the *env* coding region of MoLV, specifically missing sequences homologous to the *gag-pol* region. This is consistent with this RNA being the spliced envelope message analogous to the subgenomic message produced in a typical viral infection. The 6.0-kb band, which is absent from GIX$^-$, and the 5.2-kb band, which is present in GIX$^-$ at approximately the same level as GIX$^+$, are both homologous to only the *gag-pol* region of the virus, specifically missing any potential envelope coding information.

Although the 129 mouse strain is viral free in the sense that no infectious retroviral particles can be detected, it is interesting to note that all the major high molecular weight viral RNA species of a productively infected cell are also present in normal tissues, including the 8.0-kb, presumably full-length, viral RNA. It has been suggested that 129 endogenous viruses lack the capacity to express the polymerase function (41). If this is true, the defect responsible for this lack of expression is too small to be detected by this analysis and does not involve a major deletion.

The Expression of Multiple RNAs Is Coordinately Regulated. Serological analyses of a wide variety of tissues of the 129 GIX$^+$ mouse have detected gp70 molecules (8) with distinct antigenic characteristics (9) and tryptic peptide maps (20). The expression of viral RNA in several tissues of 129 mice was investigated by RNA blotting, revealing a tissue-specific pattern of RNA accumulation (Fig. 4*A*). The polyadenylylated RNA of whole murine brain contains barely detectable levels of endogenous retroviral RNA. Liver, spleen, thymus, and epididymis all share the 8.0-kb and 7.2-kb full-length transcripts and the 3.5-kb envelope-specific subgenomic transcript. In addition, each tissue reveals specific qualitative and quantitative differences in endogenous viral transcripts. Liver has a doublet approximately 4.1 kb long and greatly reduced amounts of the 5.2-kb band. Thymus shows reduced amounts of the full-length transcripts but accumulates a large abundance of 5.0- to 5.2-kb molecules. Spleen accumulates primarily the 5.2-kb transcript. All these tissue-specific RNA transcripts contain sequences homologous with the viral *gag-pol* region but lack any detectable homology with *env* (data not shown). Analogous transcripts were not detected in virally infected cells (Fig. 3*C*).

A similar analysis of retroviral RNA in the tissues of 129 GIX$^-$ mice revealed that all retroviral homologous transcripts in all tissues are reduced or absent in GIX$^-$ mice with the exception of the 5.2-kb band in the epididymis (Fig. 4*B*). Visualization of these RNA populations by hybridization with probes to two constitutively transcribed genes (Fig. 4*C*) provides a measure of the mass of RNA derived from each tissue. Although these results cannot confirm the finding that *distinct* tissue-specific gp70 proteins are regulated in concert, these tissue-specific RNA species, which may be transcribed from separate retroviral structural genes, are all under the control of *Gv-1*.

Some MoLV-Homologous RNA Is Regulated at the Transcriptional Level. Transcription rates of RNA sequences homologous with MoLV were measured *in vitro* by labeling na-

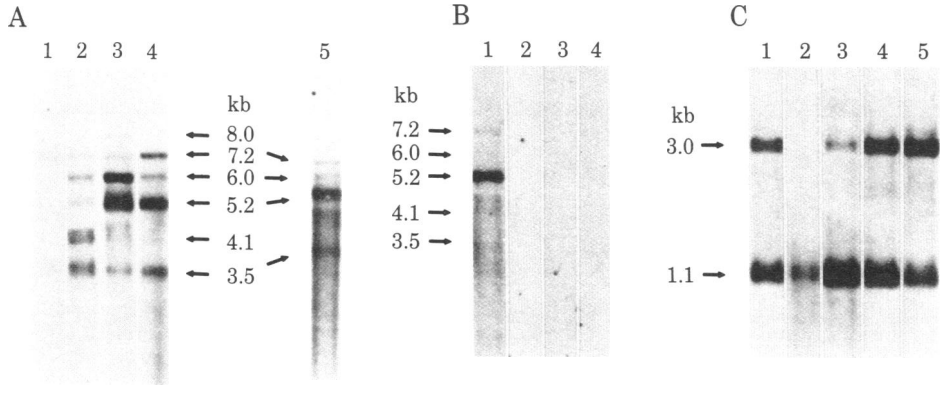

FIG. 4. Hybridization of polyadenylylated RNA from tissues of congeneic 129 mice. Polyadenylylated RNA was analyzed as described in Fig. 3. RNA from GIX$^+$ mice (*A*) and from GIX$^-$ mice (*B*) was hybridized with nick-translated pMLV-1. RNA from brain of GIX$^+$ mice and from liver, spleen, thymus, and epididymis from GIX$^-$ mice (*C*) was hybridized with nick-translated pCHO B and pCHO C. (*A*) Lanes: 1, brain; 2, liver; 3, thymus; 4, epididymus; 5, spleen. (*B*) Lanes: 1, epididymus; 2, liver; 3, spleen; 4, thymus. (*C*) Lanes: 1, brain; 2, liver; 3, spleen; 4, thymus; 5, epididymus.

scent RNA transcripts in isolated nuclei and hybridization in DNA excess. This method has been shown to provide an accurate reflection of transcription rates without the influence of processing rates and to yield an estimate similar to that obtained from pulse-labeled cells *in vivo* (32, 42). In these experiments, the nuclei of liver (a gp70-positive tissue in the GIX$^+$ strain) and brain (gp70-negative) tissue were used to provide transcription rate measurements because they are relatively easy to prepare and are highly active *in vitro*. Similar results were obtained with GIX$^+$ and GIX$^-$ spleen nuclei but at much reduced levels of incorporation (data not shown). The transcriptional activities of retroviral sequences in GIX$^+$ and GIX$^-$ mice were compared by using a probe to promotor proximal sequences at the 5′ end of the viral genome. The cDNA recombinant plasmid pCHO B was included to provide an internal standard of labeling and relative hybridization efficiency.

Comparison of the results obtained from liver nuclei from GIX$^+$ and GIX$^-$ mice (Table 1) shows that the transcription rate for *gag-pol* sequences is reduced more than 80% in the GIX$^-$ liver as compared with GIX$^+$, in agreement with the lack of mRNA observed at steady state. Moreover, transcription of retroviral sequences in brain of either GIX$^+$ or GIX$^-$ mice is at the level of GIX$^-$ liver, consistent with the reduced RNA and protein levels detected in these tissues. The transcription rate of sequences homologous with pCHO B remains constant regardless of the choice of tissue and strain of mouse, consistent with the results obtained previously (42, 43).

To investigate further the nature of the residual transcriptional activity of retroviral sequences in tissues showing little accumulation of poly(A)-containing *env* RNA, the transcription of these sequences was measured in GIX$^-$ liver in the presence of α-amanatin, a potent inhibitor of RNA polymerase II (44). Treatment of nuclei with α-amanatin during labeling reduced the total incorporation of UTP to 68% of the incorporation into untreated nuclei, consistent with a significant inhibition of RNA polymerase II activity (data not shown). Transcription of *gag-pol* sequences was reduced by 77%, showing that the majority of this synthesis is directed by RNA polymerase II. A similar analysis using a 3′ probe of *env* sequences was not possible because of a high background of α-amanatin-insensitive transcription that obscured polymerase II-directed *env* synthesis.

DISCUSSION

The mechanisms controlling the expression of specific gene products, as illustrated by animal viral systems, can occur at many steps during mRNA biogenesis, including at the level of transcription and post-transcriptional processing (for review, see ref. 45). However, the genetic loci and the gene products that dictate these mechanisms of eukaryote gene regulation remain undefined. The murine strain 129 congeneic pair is an example of the control of a particular phenotype by a single genetic locus. The GIX phenotype in 129 mice is dependent on the presence of a positive allele at *Gv-1* (4). Contrary to some suggestions (3, 18), analysis of the genomic DNA of these mice indicates that *Gv-1* is not a viral structural gene. No differences were seen in the number or location of endogenous retroviral genomes homologous with MoLV nor in the structure or number of the polyadenylylated RNA transcripts produced by these genomes. Instead, quantitative differences in the accumulation of these mRNAs were found to correlate with the GIX phenotype. Although models involving an altered genomic structure or missing provirus in 129 GIX$^-$ or invoking translational or post-translational defects could have been envisaged, the lack (or scarcity) of gp70 and p30 in GIX$^-$ mice is the result of a substantial reduction in the accumulation of mRNA transcribed from the same complement of endogenous retroviral sequences. This control by the GIX locus is mediated at the level of transcription.

The tissue-specific polymorphism exhibited by the products of endogenous retroviral sequences suggests that more than one transcription unit is affected to produce the GIX phenotype. This polymorphism is detected in two ways. First, serological (9) and structural (20) studies of gp70 proteins have shown that distinct molecular species are deposited in different tissues. Although the analyses reported here cannot detect this polymorphism because of the heterologous nature of the probe and the not unexpected similarity in size of *env* mRNAs, all potential gp70-coding mRNAs are reduced in all tissues of the GIX$^-$ strain. Second, the tissue-specific distribution of distinct polyadenylylated RNAs indicates further the activity of multiple *gag-pol* transcription units. An alternative interpretation, which, however, would not address the observed polymorphism of gp70, is that the tissue-specific pattern of *gag-pol* RNAs is the result of tissue-specific post-transcriptional processing of transcripts originating from a single *Gv-1*-regulated transcription unit. The presence of multiple copies of endogenous retroviral DNA sequences integrated within the murine genome has been well documented (refs. 1, 35, and 36; Fig. 2). Analysis by restriction enzyme mapping suggests that many of these sequences may reflect partially deleted or otherwise altered proviruses (35, 36). Tissue-specific transcription of such distinct retroviral sequences would result in the observed heterogeneity of *gag-pol* polyadenylylated RNAs. In this model, the gp70 coding sequences, whose polymorphism is defined at the protein level, would reside within these tissue-specific transcription units. Transcriptional control by *Gv-1* of these distinct multiple retroviral genomes would result in the observed down

Table 1. Transcription of MoLV-homologous RNA in GIX$^+$ and GIX$^-$ liver and brain nuclei

	GIX$^+$						GIX$^-$					
	Input, cpm × 10^{-6}		Hybridization, cpm		Transcription rate,	% GIX$^+$ liver	Input, cpm × 10^{-6}		Hybridization, cpm		Transcription rate,	% GIX$^+$ liver
Clone	Exp. 1	Exp. 2	Exp. 1	Exp. 2	no. × 10^6	rate	Exp. 1	Exp. 2	Exp. 1	Exp. 2	no. × 10^6	rate
					Liver nuclei							
pMo$^{gag\text{-}pol}$	11.5	11.5	1,356	1,706	133.1	—	11.9	9.2	297	213	24.0	18
pCHO B	11.5	11.5	260	223	21.0	—	11.9	9.2	271	147	19.4	92
					Brain nuclei							
pMo$^{gag\text{-}pol}$	3.5	1.8	72	44	22.4	17	6.7	1.4	54	40	17.9	13
pCHO B	3.5	1.8	33	40	15.7	75	6.7	1.4	84	42	20.8	99

Sucrose-purified nuclei from the livers or brains of 4- to 6-wk-old male 129 GIX$^+$ and 129 GIX$^-$ mice were incubated with [α-^{32}P]UTP as described (31, 33). Transcription rates represent means for two separate nuclei preparations of the amount of radioactivity bound to duplicate filters, as pancreatic ribonuclease-resistant hybrids, minus background per 10^6 input cpm. All results were more than twice background. The eukaryotic DNA sequence of pMo$^{gag\text{-}pol}$ is 5.5 kb long and that of pCHO B is 0.7 kb long.

regulation of all viral RNA. In this sense, the epididymal-specific 5.2-kb *gag-pol* RNA would result from an unusual transcription unit that is independent of the *Gv-1* regulatory element but does not contain *env* sequences.

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1. Rowe, W. P. (1978) *Harvey Lect.* **71,** 173–192.
2. Levy, J. A. (1978) *Curr. Top. Microbiol. Immunol.* **79,** 111–213.
3. Strand, M., Lilly, F. & August, J. T. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3682–3686.
4. Stockert, E., Old, L. J. & Boyse, E. A. (1971) *J. Exp. Med.* **133,** 1334–1355.
5. Obata, Y., Ikeda, H., Stockert, E. & Boyse, E. A. (1975) *J. Exp. Med.* **141,** 188–197.
6. Tung, J.-S., Vitetta, E. S., Fleissner, E. & Boyse, E. A. (1975) *J. Exp. Med.* **141,** 198–205.
7. Tung, J.-S., Fleissner, E., Vitetta, E. S. & Boyse, E. A. (1975) *J. Exp. Med.* **142,** 518–523.
8. Lerner, R. A., Wilson, C. B., Del Villano, B. C., McConahey, P. J. & Dixon, F. J. (1976) *J. Exp. Med.* **143,** 151–166.
9. McClintock, P. R., Ihle, J. N. & Joseph, D. R. (1977) *J. Exp. Med.* **146,** 422–434.
10. Del Villano, B. C. & Lerner, R. A. (1976) *Nature (London)* **259,** 497–499.
11. Del Villano, B. C., Nave, B., Croker, B. P., Lerner, R. A. & Dixon, F. J. (1975) *J. Exp. Med.* **141,** 171–187.
12. Lennox, E. S., Lowe, A. D., Cohn, J. & Evan, G. (1981) *Transplant. Proc.* **13,** 1759–1761.
13. Roman, J. M., Hirsch, J., Readhead, C., Levy, D., DeOgny, L. & Dreyer, W. J. (1981) *Transplant. Proc.* **13,** 1782–1786.
14. Stockert, E., Boyse, E. A., Sato, H. & Itakura, K. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2077–2081.
15. Stockert, E., Boyse, E. A., Obata, Y., Ikeda, H., Sarker, N. H. & Hoffman, H. A. (1975) *J. Exp. Med.* **142,** 512–517.
16. Rosner, M. R., Tung, J.-S., Hopkins, N. & Robbins, R. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6420–6424.
17. Donis-Keller, H., Rommelaere, J., Ellis, R. W. & Hopkins, N. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1642–1645.
18. Tung, J.-S., Shen, F.-W., Viamontes, G., Palladino, M. & Fleissner, E. (1982) *Immunogenetics* **15,** 103–108.
19. Obata, Y., Stockert, E., DeLeo, A. B., O'Donnel, P. V., Snyder, H. W. & Old, L. J. (1981) *J. Exp. Med.* **154,** 659–675.
20. Elder, J. H., Jensen, F. C., Bryant, M. L. & Lerner, R. A. (1977) *Nature (London)* **267,** 23–28.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
22. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
23. Wilson, M. C. & Darnell, J. E. (1981) *J. Mol. Biol.* **148,** 231–251.
24. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
25. Rave, N., Crkvenjakov, R. & Boedtker, H. (1979) *Nucleic Acids Res.* **6,** 3559–3567.
26. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
27. Nevins, J. R. & Wilson, M. C. (1981) *Nature (London)* **290,** 113–118.
28. Berns, A. J. M., Lai, M. H. T., Bosselman, R. A., McKennett, M. A., Bacheler, L. T., Fan, H., Maandag, E. C. R., Van der Putten, H. & Verma, I. M. (1980) *J. Virol.* **36,** 254–263.
29. Shinnick, T. M., Lerner, R. A. & Sutcliffe, J. G. (1981) *Nature (London)* **293,** 543–548.
30. Harpold, M. M., Evans, R. M., Salditt-Georgieff, M. & Darnell, J. E. (1979) *Cell* **17,** 1025–1035.
31. Clewell, D. B. & Helinski, D. R. (1970) *Biochemistry* **9,** 4428–4440.
32. Evans, R. M., Fraser, N., Ziff, E., Weber, J., Wilson, M. & Darnell, J. E. (1977) *Cell* **12,** 733–739.
33. McKnight, G. S. & Palmiter, R. D. (1979) *J. Biol. Chem.* **254,** 9050–9058.
34. Groudine, M., Peretz, M. & Weintraub, H. (1981) *Mol. Cell. Biol.* **1,** 281–288.
35. Chan, H. W., Bryan, T., Moore, J. L., Staal, S. P., Rose, W. P. & Martin, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5779–5783.
36. Buckler, C. E., Hoggan, M. S., Chan, H. W., Sears, J. F., Khan, A. S., Moore, J. L., Hartley, J. W., Rose, W. P. & Martin, M. A. (1982) *J. Virol.* **41,** 228–236.
37. Lowy, D. R., Rands, E., Chattopadhyay, S. K., Garon, C. F. & Hager, G. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 614–618.
38. Chattopadhyay, S. K., Lander, M. R., Rands, E. & Lowy, D. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5774–5778.
39. Pinter, A., Lieman-Hurwitz, J. & Fleissner, E. (1978) *Virology* **91,** 345–351.
40. Faller, D. V., Rommelaere, J. & Hopkins, N. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2964–2968.
41. Jongstra, J. & Moroni, C. (1981) *J. Virol.* **37,** 1044–1050.
42. Derman, E., Krauter, K., Walling, L., Weinberger, C., Ray, M. & Darnell, J. E. (1981) *Cell* **23,** 731–739.
43. Soreq, H., Harpold, M. H., Wilson, M. C. & Darnell, J. E. (1980) *Biochem. Biophys. Res. Commun.* **92,** 485–491.
44. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G. & Rutter, W. J. (1970) *Science* **170,** 447–449.
45. Darnell, J. E., Ziff, E., Nevins, J. R. & Wilson, M. C. (1980) *Ann. N.Y. Acad. Sci.* **354,** 466–471.